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Immunogenicity of a hybrid *Plasmodium falciparum* malaria antigen

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SUMMARY

A recombinant baculovirus-expressed hybrid protein containing epitopes for the C-terminal fragment of the *Plasmodium falciparum* precursor to the major merozoite surface antigens (PMMSA) and the tetrapeptide repeats of the circumsporozoite protein (CSP) was assessed for its immunogenicity. Murine MHC-II restriction of the antibody response to the CSP repeats was not overcome by the PMMSA component, the response to which showed no restriction. In an adjuvant trial the highest antibody titres in rabbits to both components of the hybrid were obtained using Freund's adjuvant. Lack of a boosting antibody response to the CSP repeats appeared to be linked to the conformation of the PMMSA component. Formulation of the hybrid protein into Iscoms gave antibody titres of only short duration to both components.

Key words: baculovirus, hybrid antigen, circumsporozoite protein, malaria, antigens, adjuvants, Iscoms.

INTRODUCTION

The possibility of vaccinating against malaria has led to an intensive effort aimed at identifying and characterizing malaria proteins implicated in a protective host response, resulting in several clinical trials of candidate *Plasmodium falciparum* malaria vaccines in both primates and humans with varying degrees of success (Patarroyo *et al.* 1988; Rodriguez *et al.* 1990; Ruebush *et al.* 1990; Etlinger *et al.* 1991; references in Murphy *et al.* 1990).

A candidate *P. falciparum* malaria vaccine $SC_{26}42$, a hybrid protein containing 26 tetrapeptide repeats from the immunodominant sporozoite surface antigen, the circumsporozoite protein (CSP) and the 293 amino acid C-terminal 42 kDa fragment of the precursor to the major merozoite surface antigens (PMMSA) has been expressed in *E. coli* (Holder, Lockyer & Hardy, 1988) and in insect cells using baculovirus vectors (Murphy *et al.* 1990). The latter system was exploited because it allowed the expression of conformational determinants constrained by disulphide bonds which are not maintained in *E. coli*, and which may be important in protective immunity.

In this study we have examined the immunogenicity of the baculovirus-expressed hybrid protein with different adjuvants including its assembly into Iscoms, and the possible carrier role of the merozoite component in overcoming genetic restriction to the CSP repeats.

MATERIALS AND METHODS

Construction of a hybrid gene to express both CSP and PMMSA Plasmodium falciparum *sequences and expression in insect cells*

These methods have been described previously (Holder *et al.* 1988; Murphy *et al.* 1990). The transfer vector $pSC_{26}42$ encodes a polypeptide, $SC_{26}42$, of approximately 55 kDa, constituting essentially the PMMSA signal peptide(S), 26 NANP repeats from the CSP (C_{26}) and the C-terminal 293 amino acids (42) of PMMSA (Wellcome strain). In the vector $pSC_{26}42\Delta A$, a linker containing translational stop codons upstream of the putative hydrophobic membrane anchor coding sequence allows secretion of the expressed anchor-deleted antigen, $SC_{26}42\Delta A$, from infected insect cells (Murphy *et al.* 1990). $S42\Delta A$ is encoded by a baculovirus vector lacking the 26 CSP repeats. An antigen of about 62 kDa expressed in *E. coli*, trpE-$C_{26}42$ in which the signal sequence is replaced by 116 amino acids of the *E. coli* trpE protein, was used in part of this work (Holder *et al.* 1988).

Antigen purification

Spodoptera frugiperda (Sf) cells (IPLB Sf21) were grown in suspension cultures as previously described (Murphy *et al.* 1990). Recombinant viruses were scaled up from plaques and grown on a large scale in suspension on roller bottle cultures (Summers & Smith, 1987).

Four days after infection, $pSC_{26}42$-infected Sf cells were harvested by centrifugation. Protein was

extracted using 1% *n*-octyl gluc side (OG, Sigma), in 25 mM Tris, pH 8·0, with protease inhibitors (10 μg/ml chym statin; 5 mM EDTA; 100 μg/ml leupeptin; 10 μg/ml pepstatin; 0·1 mM PMSF). After centrifugation at 10000 *g* f r 20 min the supernatant fraction was loaded ont a Mab 111.4 affinity column (coupled at 5 mg IgG/ml swollen Sepharose 4B; Holder *et al.* 1987) equilibrated with 1% OG in 25 mM Tris, pH 8·0. The column was washed with the same buffer, followed by a second wash with 1% OG in 25 mM Tris, pH 8·0, 500 mM NaCl. $SC_{26}42$ was eluted with 1% OG in 0·1 M citrate, pH 2·5 and neutralized by addition of 1 mM Tris, pH 8·8. The protein was concentrated by ultrafiltration (Amicon). The anchor-deleted antigen, $SC_{26}42\Delta A$, was purified from culture supernatants of $SC_{26}42\Delta A$-infected Sf cells 4 days post-infection. A sample of 200 ml of culture supernatant was loaded onto a Mab 111.4 affinity column as above but pre-equilibrated with phosphate-buffered saline (PBS), 0·05% Tween 20, 0·02% NaN_3. The column was washed with 200 ml of PBS, 0·05% Tween 20, and the antigen eluted with 8 M urea, 20 mM *bis*-Tris-propane, pH 7·2. Eluted antigen fractions were checked for purity by SDS–PAGE analysis, transfer to nitrocellulose and probing with Mab 111.4. $S42\Delta A$ was purified using the same protocol as for $SC_{26}42\Delta A$.

The *E. coli*-expressed antigen trpE-$C_{26}42$ was purified as previously described (Holder *et al.* 1988).

Coupling of P. falciparum *CSP-peptide to ovalbumin*

Synthesis of the peptide sequence (5-acetamidomethyl) cysteine–$(Asn\text{-}Ala\text{-}Asn\text{-}Pro)_4$–Asn-Ala has been described previously (Holder *et al.* 1988). A total of 49 μmole (2·25 mg) ovalbumin was coupled to 1·1 μmole (2·25 mg) peptide in 500 μl of 0·05 M sodium phosphate, pH 7·8 by dropwise addition of 125 μl of 0·2% glutaraldehyde solution in the same buffer over 1 h with stirring. The mixture was stirred for a further hour and dialysed overnight against PBS.

Production of monoclonal antibody 300.1

C57BL/6 mice were immunized 3 times with 50 μg $(NANP)_4$–BSA conjugate. The first 2 immunizations were intraperitoneally in Freund's complete adjuvant (FCA) and the third intravenously in Freund's incomplete adjuvant (FIA). Spleen cells from immunized mice were fused with P3-x63-Ag8-653 myeloma cells (BALB/c) 3 days after the final immunization as previously described (Kearney *et al.* 1979; Crowe *et al.* 1984). Fusion supernatants were screened by ELISA. PVC microtitre plates were coated with CSP peptide–ovalbumin and serial dilutions of the fusion supernatants added. After incubation and washing, antibody binding was detected with anti-mouse IgG conjugated to h rseradish peroxidase, addition of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate and measurement of absorbance at 450 nm. Positive Mabs were screened a second time using microtitre plates coated with CSP peptide alone and finally with $SC_{26}42\Delta A$. One Mab, 300.1, was used for measurements of CSP repeat antibody titres.

Adjuvant studies

The antigen was emulsified with 0·5 ml of FCA (Sigma) or mixed with 250 mg saponin or 250 mg Alhydrogel (Superfos) in a final volume of 1 ml for each dose. Pluronic droplets were prepared by emulsifying 10% (v/v) squalene and 5% (v/v) Pluronic L121® in PBS containing 0·4% Tween 80. For each dose, 100 μg antigen in 0·5 ml PBS/0·4% Tween 80 was mixed with an equal volume of emulsion. Syntex Adjuvant Formulation (SAF, Allison & Byars, 1986) was prepared by the addition of 0·25 ml of threonyl–MDP to the antigen in PBS/0·4% Tween 80 prior to mixing with pluronic droplets.

Iscoms

Iscoms containing $SC_{26}42$ were prepared by mixing cholesterol/phosphatidyl choline (10 mg/ml cholesterol + 10 mg/ml phosphatidyl choline in 20% (v/v) Mega 10) and QuilA (10 mg/ml) (gifts from Dr B. Sunquist, Iscotech) in a 1 : 1 molar ratio with 20 μl of 1·2 mg/ml $SC_{26}42$ and 80 μl of Mega 10, and dialysed overnight against 50 mM Tris, pH 7·2, 100 mM NaCl (TN).

Iscoms were fractionated by centrifugation in sucrose gradients. Samples of 50 μl vol. were loaded onto 10–50% gradients in TN buffer and centrifuged in a Beckman SW50 rotor 4 h at 150000 *g*. Iscoms incorporating $SC_{26}42$ were localized in the fractionated gradients by ELISA using either the CSP repeat Mab 300.1 or polyclonal rabbit $S42\Delta A$ antiserum for detection. The particles had a typical Iscom morphology as shown by scanning electron microscopy and a sedimentation coefficient of 19S.

Antisera

For the adjuvant study rabbits (2/group) were immunized intramuscularly with 100 μg of $SC_{26}42\Delta A$, while a third animal received adjuvant alone, with further immunizations after 28 and 49 days. Serum samples were collected 14, 35 and 63 days after the first immunizati n. Animals which received FCA in the first injecti n subsequently received FIA. In the follow-up study rabbits were

Table 1. Murine T-cell proliferative respons to $SC_{26}42\Delta A$

(Mice were immunized subcutaneously in the tail base with 50 μg antigen in FCA alone. After 10 days the proliferative response was measured as described in the Materials and Methods section. The Stimulation Index (SI) was calculated as the mean cpm of duplicate antigen-stimulated lymph node cell cultures divided by the mean cpm of unstimulated controls (−).)

		Cpm incorporation *in vivo*					
		Immune LNC			Control LNC		
Mouse strain	H-2	42 (SI)	NANP (SI)	−	42 (SI)	NANP (SI)	−
C57BL/6	b	48653 (7·0)	35726 (5·1)	6976	9318 (2·1)	5653 (1·3)	4530
B10.S	s	81060 (5·8)	12136 (0·9)	4090	8686 (1·6)	5271 (1·0)	5532
B10.D2	d	89786 (15·1)	7611 (1·3)	5942	5568 (2·5)	2713 (1·2)	2261
CBA/T6T6	k	141456 (16·1)	14518 (1·7)	8772	49254 (2·5)	25846 (1·3)	20046

immunized using Freund's and a similar schedule with either $SC_{26}42\Delta A$ (baculovirus), trpE-$C_{26}42\Delta A$ (*E. coli*) or 100 μg CSP peptide–ovalbumin.

For the dose–response study using Iscoms, 5 groups of 4 C57BL/6 mice were immunized subcutaneously with 0·1, 1, 3, 5 or 20 μg $SC_{26}42$/Iscoms with booster immunizations after 1 week. Serum samples were taken 1 week after each immunization and assayed by ELISA for antibody responses to the hybrid. In rabbit immunization studies, groups of 2 animals received 25 μg $SC_{26}42$/Iscoms intramuscularly with a boost after 28 days. Control animals received the equivalent amounts of QuilA/cholesterol mix without antigen, and a third group $SC_{26}42$ in FCA (boosting was in FIA). Serum samples were taken every 2 weeks for 12 weeks.

Analysis of the antibody response

The serum antibody response was monitored by ELISA. PVC microtitre plates were coated with either CSP peptide or $S42\Delta A$ and serial dilutions of the antisera added. After incubation and washing, antibody binding was detected with anti-rabbit IgG coupled to horseradish peroxidase IgG (Sigma) and TMB substrate and A450 values read.

T-cell proliferation studies

B10.S and CBA/T6T6 mice were bred in our own animal facility. C57BL/6 and B10.D2 mice were btained from OLAC. Groups of 2 mice were immunized subcutaneously in the base of the tail with 50 μg $SC_{26}42\Delta A$ in FCA or with FCA alone. Ten days later lymph nodes were removed and duplicate single cell suspensions made. Clicks EHAA medium supplemented with 2 mM L-glutamine, 100 iu/ml penicillin/streptomycin, 5×10^{-6} M β-mercaptoethanol and 0·5% normal mouse serum was used for *in vitro* culture. Lymph node cells (4×10^5) were stimulated with antigen ($S_{42}\Delta A$ or CSP peptide) at a final concentration of 12·5 μg/ml in a volume of 200 μl. DNA synthesis was measured by [^{3}H]thymidine incorporation after 3 days of culture.

RESULTS

Restriction of the murine T-cell response to $SC_{26}42\Delta A$ in inbred strains

Table 1 shows the proliferative response of 4 inbred mouse strains to each component of the hybrid protein. The strain C57BL/6 with the MHC II haplotype $H2^b$ was the only strain showing a significant response to the NANP repeats of the CSP (Stimulation Index [SI] = 5·1). In contrast all the tested strains responded to the PMMSA component, although there was a 3-fold variation observed in the degree of response (SI = 5·8–16·1).

Antibody response to $SC_{26}42\Delta A$ with different adjuvants

The hybrid protein was used to immunize rabbits in an adjuvant study and the antibody response to each component measured (Fig. 1). All animals made a primary response to both $S42\Delta A$ and the CSP repeats (three animals 716, 712 and 706, one from each of the groups receiving pluronic droplets, SAF and saponin respectively, gave a poor anti-$S42\Delta A$ response to the first immunization; 716 and 712 also responded poorly to the CSP component). There was a boosting response to $S42\Delta A$ with all adjuvants after the second immunization (Fig. 1A), which was not observed with the CSP repeats, where the titres fell over the subsequent 7 weeks. The CSP repeat titres of the animals receiving the antigen with pluronic droplets and one animal (712) receiving SAF showed a m dest boosting response after the second immunization but the titres subsequently fell as with the other adjuvants. Of the adjuvants tested,

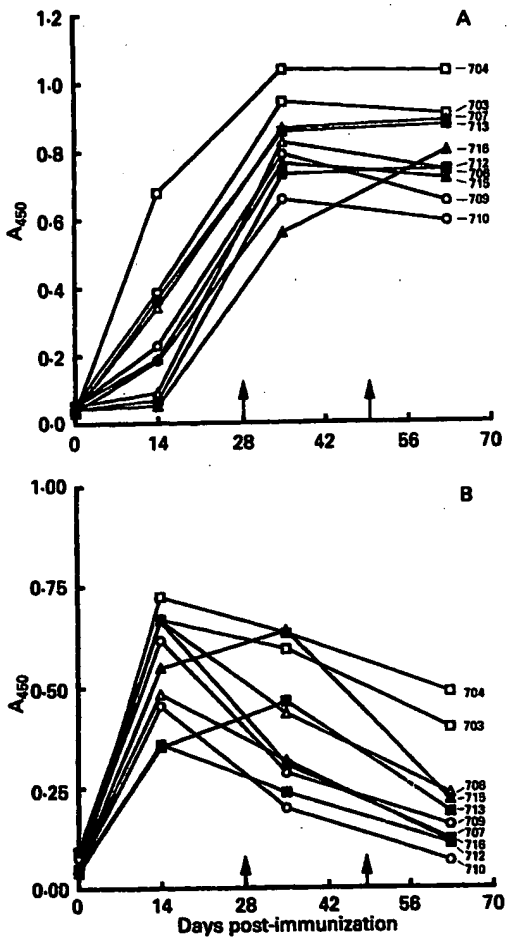

Fig. 1. Antibody response of rabbits to $SC_{26}42\Delta A$ with different adjuvants measured by ELISA. (A) Response to $S42\Delta A$; (B) response to C_{26} using $S42\Delta A$ and CSP–ovalbumin conjugate respectively to coat the microtitre plates. The A_{450} ELISA values are the mean of duplicate readings. The data represent 1 in 3200 serum dilution values. Arrows indicate the time of booster immunizations. (□) Freund's; (△) saponin; (■) SAF; (▲) pluronic droplets; (○) Alhydrogel.

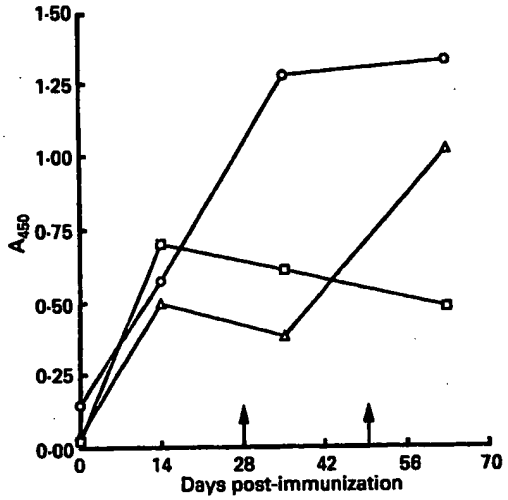

Fig. 2. Comparison of the CSP repeat antibody response of rabbits using hybrid antigen expressed in baculovirus ($SC_{26}42\Delta A$) and *E. coli* (trpE-$C_{26}42$). The A_{450} ELISA figures are the mean of duplicate values for 2 rabbits at each time-point after immunization using CSP–ovalbumin as the capture antigen. The data represent 1 in 3200 serum dilution values. Arrows indicate the time of booster immunizations. (□) $SC_{26}42\Delta A$ from baculovirus; (△) trpE-$C_{26}42$ from *E. coli*; (○) CSP–ovalbumin conjugate.

Freund's gave the best antibody response against $S42\Delta A$, with Alhydrogel and pluronic droplets the poorest. There was greater individual variation within each adjuvant group in the CSP repeat responses, but the highest titres against the latter were again obtained using Freund's. The peak titre values against both components of $SC_{26}42\Delta A$ were greater than 1 in 12000.

An approach to understanding the basis for the lack of boosting to the CSP component of the hybrid protein was made in a second series of experiments. Rabbits were immunized with either $SC_{26}42\Delta A$ (baculovirus), trpE-$C_{26}42$ (*E. coli*) (Holder *et al.* 1988) or with the CSP repeat–ovalbumin peptide conjugate alone, using the same schedule to that described above. Fig. 2 shows that the lack of boosting of the anti-CSP repeats response observed with the baculovirus-expressed protein did not occur with the hybrid protein expressed in *E. coli* although there was initially a similar fall in antibody titre prior to the second immunization. There was also a normal boosting response to the CSP repeats alone. All animals immunized with $SC_{26}42\Delta A$ or trpE-$C_{26}42$ gave a normal boosting response to $S42\Delta A$ (not shown).

The antibody responses to both the sporozoite and merozoite components of mice immunized with $SC_{26}42$/Iscoms were barely detectable with no boosting and no dose–response effect (not shown). In rabbits boosting responses were obtained to both components (Fig. 3). One animal (812) showed a significantly higher antibody response to each component but the pattern of the S42 response was similar in both animals; animal 811 responded poorly to the CSP repeats. The magnitude of the peak antibody response of animal 812 to both components was similar to that of animals which received antigen with Freund's adjuvant but the titres of both animals receiving $SC_{26}42$/Iscoms were not maintained and began to fall 6 weeks post-immunization. It is also apparent from the results of Fig. 3 that, in contrast to $SC_{26}42\Delta A$, the presence of the PMMSA C–terminal anchor on the antigen allowed normal boosting of the response to the CSP repeats in animals immunized with Freund's adjuvant.

Lymphoproliferative studies showed that T-cells from C57BL/6 mice immunized with $SC_{26}42$/

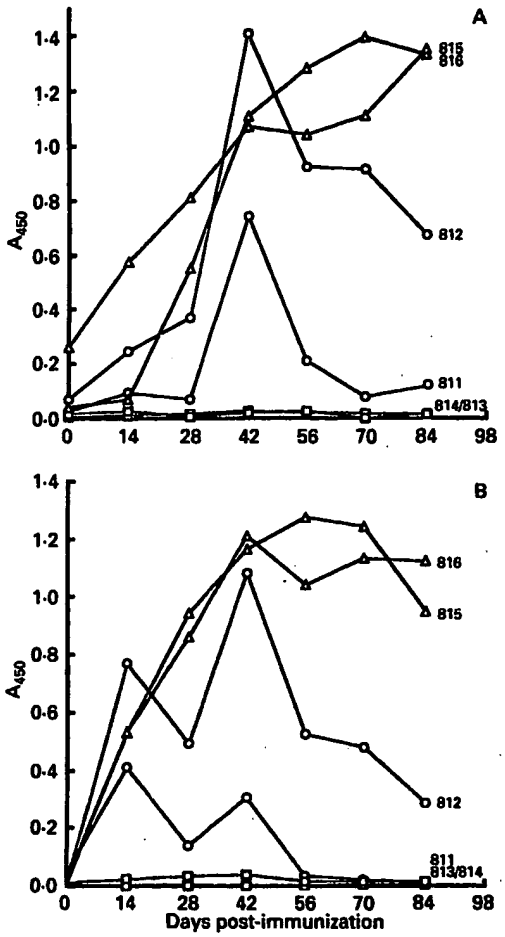

Fig. 3. Antibody response to $SC_{26}42$ in Iscoms. Groups of 2 rabbits were immunized with $SC_{26}42$/Iscoms (○) as described in the Materials and Methods section. The responses to Iscoms with no incorporated antigen (□) and $SC_{26}42$ in Freund's adjuvant (△) are also shown. The A_{450} ELISA values are the mean of duplicate readings at a 1 in 1250 dilution using S42*ΔA* (A) or CSP–ovalbumin conjugate (B) as the capture antigen. Arrow indicates the time of booster immunization.

Iscoms proliferated upon challenge with S42*ΔA* (SI = 13) a similar degree of stimulation observed with $SC_{26}42$/Freund's (SI = 12·5). By contrast there was no detectable proliferative response to the CSP repeats using $SC_{26}42$/Iscoms where an SI of 9 was obtained with the antigen in Freund's (not shown).

DISCUSSION

A hybrid merozoite/sporozoite recombinant malaria antigen expressed by baculovirus in insect cells has been shown to elicit an antibody response in rabbits, and a lymphoproliferative T-cell response in mice. One reason for designing such a hybrid was the possibility that the PMMSA sequence might act as a carrier molecule for the CSP sequence in genetically non-responder animals. However, the response to the CSP repeats was restricted to H-2^b mice as previ usly shown for the repeats alone (Del Giudice *et al.* 1986; Togna *et al.* 1986; Good *et al.* 1986). The anti-42 response showed no MHC II restriction which has also been demonstrated for purified PMMSA (Chang *et al.* 1989).

Inclusion of the CSP T-cell epitope Th2R (Good *et al.* 1987) in the antigen might overcome restriction of the CSP response, as was observed in a study using a hybrid CSP/liver-stage synthetic peptide molecule (Londono *et al.* 1990). However, the heterogeneity in this region in parasite populations probably precludes this option (Lockyer, Marsh & Newbold, 1989). A second possibility is the addition of a cysteine residue to the N-terminus of the CSP repeats which Rzepczyk *et al.* (1990) showed capable of overcoming H-2^b restriction.

The adjuvant study in rabbits underlined the superiority of Freund's over the other adjuvants tested in the response to the PMMSA component, and the results emphasize the need for an adjuvant with the efficacy of Freund's for human use. The significant variation observed in the magnitude of the antibody responses of animals within some groups, particularly against the CSP repeats probably reflects the fact that the rabbits are outbred.

The lack of boosting of the anti-CSP repeat response appears to be related to the conformation of the PMMSA component. Previous experiments showed that certain Mabs recognizing the C-terminal 42 kDa PMMSA fragment fail to recognize the reduced antigen but that this recognition is restored under non-reducing conditions where the correct disulphide bonds can form (Holder *et al.* 1988). Murphy *et al.* (1989) showed that these conditions are fulfilled with the baculovirus expression system used in these experiments. However, the hybrid antigen expressed in the reducing environment of *E. coli*, where the correct conformation is not adopted, elicited a boosting antibody response to the CSP repeats after a small drop in titre. It is possible that the 'correctly' folded PMMSA component exerts an intramolecular suppressive effect on the response to the CSP repeats, perhaps due to steric hindrance rendering the repeats relatively inaccessible. This is supported by the observation that in quantitative ELISA assays capture of the PMMSA component of $SC_{26}42\Delta A$ by the conformation-specific Mabs 111.4 or 111.2 (Holder *et al.* 1988) precluded detection of the CSP component by Mab 300.1. This problem was also observed when 300.1 served as the capture Mab and Mab 111.4 or 111.2 was used for detection (data not shown). It may also be possible to circumvent this problem either by reversing the order of the hybrid (i.e. $S42C_{26}\Delta A$) or by separately immunizing with each component of the hybrid. The latter, of course, confounds the original advantage of purifying a single, bi-functional antigen. Interestingly, the ad-

diti n of a hydr phobic anchor als overcame the suppressive effect but the yield of this pr tein, which is not secreted, was much lower than that of the anchor-deleted hybrid.

The reasons for the poor immunogenicity of $SC_{26}42$/Iscoms in mice are not clear. The C57BL/6 strain is a responder strain for an anti-NANP response and we have shown that there is no MHC Class II restriction of the anti-42 response. The poor duration of antibody responses to the hybrid antigen in Iscoms was both surprising and unexpected, since their adjuvant properties have already been demonstrated for various viral and microbial antigens, both in terms of immunogenicity and protective efficacy (Morein, Lovgren & Hoglunds, 1989). Although the above experiment did not allow testing for protection, which may rely on cell-mediated immune mechanisms, C57BL/6 mice immunized with $SC_{26}42$/Iscoms failed to show a lymphoproliferative T-cell response to the CSP repeats and the response to the PMMSA component was also poor (data not shown). ELISA monitoring of Iscoms showed that both Mab 111.4 and 300.1 recognized the hybrid protein in Iscoms, and both their 19S value and ultrastructural morphology were consistent with correct Iscom structure. Since $SC_{26}42$ in Freund's adjuvant produced titres of longer duration it appears that the packing of the protein into the Iscom has a deleterious effect on the immunogenicity of both components. The problem may again be related to antigen conformation and it would be interesting to ascertain whether immunization of animals with Iscoms containing only one component would give higher titres and improved T-cell responses. More work is clearly needed to elucidate further and resolve the problems in the present study, before the hybrid antigen can be considered for use as a human vaccine.

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☐ 1: P04933 **MEROZOITE SURFACE PROTEIN 1 PRECURSOR (MEROZOITE SURFACE ANTIGENS) (PMMSA) (P195)**

BLink, PubMed, Related Sequences, Taxonomy, LinkOut

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            created: Aug 13, 1987.
            sequence updated: Feb 1, 1996.
            annotation updated: Feb 1, 1996.
            xrefs: gi: gi: 9864, gi: gi: 9865, gi: gi: 84241
            xrefs (non-sequence databases): PFAM PF00008
KEYWORDS    Malaria; Merozoite; Polyprotein; Repeat; Signal; Glycoprotein;
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  ORGANISM  Plasmodium falciparum (isolate WELLCOME)
            Eukaryota; Alveolata; Apicomplexa; Haemosporida; Plasmodium.
REFERENCE   1  (residues 1 to 1639)
  AUTHORS   HOLDER,A.A., LOCKYER,M.J., ODINK,K.G., SANDHU,J.S.,
            RIVEROS-MORENO,V., NICHOLLS,S.C., HILLMAN,Y., DAVEY,L.S.,
            TIZARD,M.L.V., SCHWARZ,R.T. and FREEMAN,R.R.
  TITLE     Primary structure of the precursor to the three major surface
            antigens of Plasmodium falciparum merozoites
  JOURNAL   Nature 317 (6034), 270-273 (1985)
  MEDLINE   86014355
  REMARK    SEQUENCE FROM N.A.
REFERENCE   2  (residues 1 to 1639)
  AUTHORS   HOLDER,A.A.
  TITLE     Direct Submission
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  REMARK    REVISIONS.
COMMENT     On Jun 1, 1996 this sequence version replaced gi:127335.
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            This SWISS-PROT entry is copyright. It is produced through a
            collaboration between the Swiss Institute of Bioinformatics and
            the EMBL outstation - the European Bioinformatics Institute.
            The original entry is available from http://www.expasy.ch/sprot
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            [SUBCELLULAR LOCATION] ATTACHED TO THE MEMBRANE BY A GPI-ANCHOR
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AQUILA ANNOUNCES PHASE I CLINICAL TRIAL OF A MALARIA VACCINE THAT COMBINES QS-21 ADJUVANT WITH SPf66 ANTIGEN

-Study is in collaboration with WHO-

Worcester, MA, December 1, 1997 -- Aquila Biopharmaceuticals, Inc. (NASD NMS: AQLA) announced today the initiation of a collaborative Phase I human clinical trial to evaluate the safety and immunogenicity of a promising malaria vaccine. The potential vaccine contains Aquila's proprietary immune enhancing Stimulonô adjuvant, QS-21, and the synthetic peptide antigen SPf66, developed by Dr. Manuel Patarroyo. The trial is co-sponsored by the World Health Organization (WHO) and the Colombian Government, and is being conducted under the direction of Dr. Patarroyo, at Instituto de Inmunologia, with collaborators from Aquila Biopharmaceuticals and the University of Barcelona in Spain.

"There is a critical need for development of a safe and effective vaccine to control malaria," said Dr. Howard Engers of the World Health Organization. "This trial is part of our effort to extend Dr. Patarroyo's earlier clinical research and evaluate potential vaccine enhancements. A vaccine incorporating SPf66 and QS-21 offers a promising approach to effective disease management. In preclinical studies this new combined vaccine product was superior to the SPf66 vaccine with alum, a conventional vaccine adjuvant." Dr. Engers noted that the WHO currently estimates that malaria kills between 1.5 - 2.7 million people worldwide each year, and as many as one to two billion individuals are at risk for infection annually.

"In this current SPf66/QS-21 malaria clinical trial, we hope to capitalize on the potent immune stimulatory activity of the Stimulonô adjuvant QS-21," said Alison Taunton-Rigby, Ph.D., President and Chief Executive Officer of Aquila. "This trial is part of our overall program to apply QS-21 and the Company's proprietary Stimulonô technology to the development of new improved vaccine and immunotherapeutic products."

The SPf66 antigen in combination with alum has been tested extensively in human clinical trials. Although protection from infection was seen in some trials, inconsistent results were obtained. These results suggest that a more potent adjuvant is required. Preclinical studies conducted by Aquila and Dr. Patarroyo support the benefit of a combined SPf66/QS-21 vaccine. In primate challenge trials, when SPf66 was administered in combination with QS-21, the protective efficacy rate was close to 60% as opposed to only 25% for the SPf66/alum vaccine.

In this SPf66/QS-21 Phase I trial, 87 volunteers enrolled in five groups received the SPf66 vaccine formulated with the QS-21 adjuvant, alum or various doses of QS-21 adjuvant with alum. Vaccination with three sequential subcutaneous injections will be used to determine the safety and immunogenicity of the potential product. The study is expected to be completed in approximately twelve months.

Unlike most malaria vaccines tested to date, which are based on a single antigen and target one of three life cycle stages of the malaria parasite, SPf66 is a hybrid molecule that contains antigenic domains from two parasite life cycle stages. The SPf66 vaccine has been extensively tested in human field trials in both low- and high-disease transmission areas, including South America, Africa and Southeast Asia, and to date has shown a strong safety

profile. It is partially protective when administered in formulations with alum.

Aquila Biopharmaceuticals, Inc. is a therapeutics biotechnology company located in Worcester, Massachusetts. The Company is developing and commercializing products which modulate the immune system for use in treating, controlling or preventing infectious diseases and cancers. Aquila's proprietary products include the Quilimmune™human products for pneumococcal infections, malaria and tick-borne diseases, and the Quilvax™animal vaccines for bovine mastitis, canine Lyme disease and feline leukemia. These products incorporate Aquila's Stimulon™adjuvants, which are also included in products under development by the Company's six corporate partners.

Statements in this release which relate to expectations and objectives of management for future operations of Aquila Biopharmaceuticals, Inc., or which otherwise relate to future performance are forward looking statements. Actual results may differ from those projected as a result of product demand, pricing, market acceptance, economic conditions, intellectual property issues, competitive products, risks in product and technology development and other risks identified in the Company's Securities and Exchange Commission filings.

For investor inquiries, please call (508) 797-5777 ext. 541. Aquila press releases may be found on the Internet at http://www.prnewswire.com (company news on call), or they may be requested by fax by calling (800) 758-5804 x134225.

A backgrounder on Aquila's Malaria Vaccine Development Programs is available upon request.

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Douglas MacDougall
Feinstein Kean Partners
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AMVTN The African Malaria Vaccine T sting N twork

HUMAN PHASE I / CHALLENGE VACCINE TRIALS OF 3 RECOMBINANT ASEXUAL STAGE MALARIA ANTIGENS

Allan Saul

The Queensland Institute of Medical Research. P.O. Royal Brisbane Hospital, Queensland, Australia

Two phase I vaccine trials have been conducted to test the immunogenicity and safety of a vaccine comprising three recombinant malaria antigens from the asexual stage of *P. falciparum* in Montanide ISA 720 adjuvant. The three antigens are 190L, a fragment of MSP-1; MSA-2 and portion of RESA. MSP-1 and MSA-2 are merozoite surface antigens. RESA is present in merozoites and is associated with the red cell cytoskeleton following invasion. The Montanide ISA 720 adjuvant is produced by SEPPIC and is a metabolizable oil with a mannide mono-oleate emulsifier that has given promising activity in animal trials and developed for human use. These trials investigated the dose response of each antigen for eliciting both antibody and T cell responses and the efficacy of a mixture of the antigens compared with the antigens injected separately. The responses of the MSA2 component in Montanide ISA720 were also compared with the responses obtained in an earlier trial that used alum as the adjuvant.

All three antigens elicited both antibody and T cell responses. Particularly strong T cell responses were observed with 190L and RESA. Stimulation indices exceeded 100 for peripheral blood leukocytes in some individuals. By contrast, the antibody responses, although significant, were substantially lower than had been observed in trials with small animals. The antibody responses observed with MSA2 were not significantly different in the volunteers in this trial with Montanide ISA720 adjuvant than previously observed with alum. No antigenic competition was observed: volunteers receiving a mixture of antigens had similar responses to those receiving the three antigens at separate sites.

Following the initial trials, the vaccine was tested for its ability to decrease the initial growth rates of parasites in human volunteers in a placebo controlled, double blind trial. Twelve volunteers each received two doses, 6 weeks apart and containing 13 mg of each component. Five placebos received an equivalent volume of the adjuvant emulsion. Four weeks following the second vaccination, volunteers were challenged i.v. with approximately 100 ring infected red cells of the 3D7 cloned line. Parasitaemias were determined daily from day 4 using a quantitative PCR assay [1] covers the range of 10 to 10,000 parasites per ml. All volunteers were treated on day 8, before any developed symptoms of malaria or parasites patent by microscopy. None of the vaccinees had a significant decrease in initial growth rates, nor was there any significant correlation between growth rates and any of the measured immunological responses. These results suggest that in naive recipients, formulations that result in significantly greater immunogenicity will be required before these antigens will be protective. Although the vaccine failed to show protection, the trial itself was a success since it gave a clear-cut estimate of the efficacy in this naive population. Importantly for future vaccine work, it demonstrated that the challenge system leads to reproducible infections in volunteers. We believe that this system could now be used for the testing of other asexual stage vaccines.

This work was the product of a large team of people from The Queensland Institute of Medical Research, The Royal Brisbane Hospital, The Walter and Eliza Hall Institute of Medical Research, Biotech Australian, CSL Pty Ltd, Australia, F Hoffmann La Roche, Switzerland. It was funded with commercial funding from Saramane Pty Ltd and F Hofmann La Roche and with research funds from The Australian National Health and Medical Research Council, the WHO/TDR Program and the Rotary Against Malaria Program.

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1. Cheng et al, Am J Trop Med Hyg (in press)

Presented at the 2nd AMVTN Meeting, Accra, Ghana, 24-26 November, 1997

Send your comments etc. to webmaster@amvtn.org